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Phytochemical Profile, Antioxidant, Antidiabetic, and Antidiarrheal Potentials of *Hylocereus undatus* Leaf Extract: GC–MS and In Silico Insights

Md. Tariqul Islam¹  | Sudipta Saha¹ | A. B. M. Ashraful^{1,2}  | Md. Shafiqul Islam³

¹Department of Pharmacy, R. P. Shaha University, Narayanganj 1400, Bangladesh | ²Department of Pharmacy, University of Rajshahi, Rajshahi 6205, Bangladesh | ³Department of Pharmacy, Gopalganj Science and Technology University, Gopalganj 8105, Bangladesh

Correspondence: Md. Tariqul Islam (tariqul.ru.23@gmail.com) | A. B. M. Ashraful (abmashraful98@gmail.com)

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ABSTRACT

Hylocereus undatus (Haworth) Britton and Rose is a popular and extensively used medicinal herb, especially in Asia, which encompasses several medicinal phytochemicals that possess curative properties. This study explored its antioxidant, antidiabetic, and antidiarrheal properties by gas chromatography-mass spectrometry (GC–MS)-based identification of bioactive compounds along with an in silico study. Leaf extract of *H. undatus* was prepared using *n*-hexane. In vitro antioxidant activities were evaluated using spectrophotometric methods. In vivo antidiabetic activity was determined by oral glucose tolerance test, and antidiarrheal activity was assessed via castor oil-induced diarrhea in mice. The bioactive compounds were identified via GC–MS. An in silico study was done by a computer-aided model using the α -amylase protein. The phytochemical screening exhibited a wide variety of plant metabolites. The total phenolic, total flavonoid, total tannin, and total antioxidant capacity were estimated as 29.73 ± 0.33 mg of GAE/g, 69.3 ± 6.61 mg of QE/g, 14.35 ± 0.221 mg of GAE/g, and 115.58 ± 1.43 mg of AAE/g, respectively. The OGTT test of the extract at 500 mg/kg produced a noteworthy ($p < 0.01$) reduction in BGL at 30 min (14.6%), 60 min (26.4%), 90 min (40.9%), and 120 min (49.4%) post-treatment compared to the control group. In the castor oil-induced diarrhea model, nHE (500 mg/kg, 39.58%) significantly ($p < 0.01$) delayed diarrhea onset and reduced total feces. The extent of these activities resembled that of standard loperamide (5 mg/kg, 58.33%). GC–MS identified 72 compounds, including β -amyron, lupeol, campesterol, stigmasterol, and vitamin E. In silico study also validated the potentialities of constituents of *H. undatus*. This is the first integrated study combining in vitro, in vivo, and in silico approaches to elucidate the pharmacological potential of *H. undatus* leaves, highlighting the extract as a promising source of therapeutic candidates. Further mechanistic studies are warranted.

1 | Introduction

Oxidative stress, triggered by imbalanced production of reactive oxygen species (ROS), plays a crucial role in the progression of various chronic diseases [1]. Antioxidants, particularly edible and nonedible plant materials containing

polyphenols, are substances that prevent the overproduction of ROS by supplying the electrons [2]. Therefore, antioxidant-rich plant extract is crucial for new treatment strategies, and plant extract has lower side effects compared to the synthetic drug [3].

Md. Tariqul Islam and Sudipta Saha contributed equally to this work.

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One such condition influenced by oxidative stress is diabetes mellitus, a global public health problem characterized by chronic hyperglycemia, due to defective insulin secretion, insulin resistance, or both [4]. One of the best-suited therapeutic approaches for hyperglycemic state after a meal is the inhibition of the carbohydrate-splitting enzymes, predominantly α -amylase and α -glucosidase [5]. Although synthetic inhibitors like acarbose are effective, they often cause adverse gastrointestinal effects [6]. Thus, plant-derived enzyme inhibitors are being explored as safer alternatives [7]. Another major global health concern is diarrhea, which is common in low-resource settings, that remains a major contributor to morbidity and mortality illnesses [8]. Natural remedies, particularly plant-based extracts that can modulate gut activity, represent an affordable and potentially effective therapeutic strategy [9].

Modern therapeutics increasingly benefit from the use of medicinal plants which provide compounds with diverse structures and complex pharmacological activities [10]. In this context, the Cactaceae family is known for its ethnomedicinal uses as well as for secondary metabolite production and remarkable survivability in harsh environments [11]. *Hylocereus undatus* (Haworth) Britton and Rose (*H. undatus*), well recognized as white pitaya or white-fleshed dragon fruit, is named for its distinctive white pulp. *H. undatus* originates from Mexico and has since been migrated to various Asian countries, including Taiwan, Malaysia, Bangladesh, Vietnam, and Australia [12]. The fresh flesh of pitaya contains small black seeds dispersed throughout its white pulp [13]. The raw flesh has a sugary taste but is low in calories [14].

While numerous studies have extensively investigated the fruit pulp of *H. undatus*, particularly using polar solvents to evaluate antioxidant and metabolic activities, the leaves often regarded as agro-waste remain largely unexplored scientifically. Traditionally, the leaves are used to manage gastrointestinal disorders and metabolic conditions [15]. Although these traditional claims suggest therapeutic relevance, there is currently no scientific or pharmacological evidence supporting the use of *H. undatus* leaf *n*-hexane extract (nHE) for such treatments.

Therefore, this study was designed to address this research gap by investigating the phytochemical profile, antioxidant capacity, antidiabetic, and antidiarrheal activities of the nHE of *H. undatus* leaves. Additionally, GC–MS analysis and molecular docking were employed to elucidate the chemical composition and potential mechanisms underlying the observed biological activities.

2 | Materials and Methods

2.1 | Chemicals

Aluminum trichloride, sodium carbonate, ascorbic acid (AA), ammonium molybdate, sodium hydroxide, methanol, gallic acid (GA), quercetin, Folin–Ciocalteu reagent (FCR), sodium nitrite, sulfuric acid, sodium phosphate, Tween-80, castor oil, and glucose were procured from Merck, Germany, via local authorized vendors. Glibenclamide and loperamide hydrochloride were collected from Square Pharmaceuticals Ltd, Bangladesh.

2.2 | Plant Collection

Leaves of *H. undatus* were harvested from Keraniganj, Bangladesh, in April 2024, and the species was authenticated by the

Bangladesh National Herbarium, Dhaka (Accession No.: DACB 135339). After collection, the plant material was meticulously rinsed with water to eliminate unwanted matter. It was then partially air-dried in a shaded area before oven drying at 40°C for about 48 h. The dried material was subsequently ground into a fine powder using a suitable mechanical grinder.

2.3 | Extract Preparation

The extraction was performed according to Hossain et al. [16] with minor modifications. Ground leaf powder (345 g) was immersed in 1300 mL of *n*-hexane in an amber glass bottle for 17 days, with occasional stirring [17]. *n*-Hexane was selected as a nonpolar solvent to selectively extract lipophilic phytoconstituents such as terpenoids, sterols, and fatty acids, which have been reported to contribute to antioxidant and antidiabetic activities through nonphenolic mechanisms, including modulation of oxidative stress and glucose metabolism [18]. After extraction, the mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated by solvent evaporation at room temperature using a table fan. It produced a concentrated brown hue that was termed as crude nHE. The dried extracts were stored in tightly sealed containers in a refrigerator at 4°C for further use. The percentage yield of nHE was found to be 5%.

2.4 | Phytochemical Screening

The freshly prepared nHE was analyzed qualitatively to identify and characterize various phytochemical components. Established protocols, as documented in previous studies, were utilized to screen for bioactive compounds, including carbohydrates (Molisch's test), alkaloids (Dragendorff's test), tannins (ferric chloride test), flavonoids (Shinoda test), saponins (foam test), gums (Molisch's test), steroids (Liebermann–Burchard's test), glycosides (Keller–Kiliani test), xanthoproteins (yellow protein test), phenolic compounds (FeCl₃ solution test), and acidic substances (sodium bicarbonate test) [19–21]. All assays were carried out using the prescribed reagents, and reagent blanks were included as negative controls to ensure accuracy and specificity of the tests.

2.5 | Determination of Total Phenolic Content

The total phenolics of *H. undatus* nHE were estimated using a modified procedure based on Singleton et al. [22]. We used GA as a standard to plot the calibration curve. FCR (5 mL, 10% v/v) was mixed with 0.5 mL of the extract sample in a test tube, followed by vortexing for 15 s to ensure proper mixing. Afterward, 4 mL of a 7% (w/v) sodium carbonate solution was added and allowed to complete the reactions at 40°C for 30 min. The extract was evaluated at 1000 µg/mL concentration. The absorbance was then recorded at 765 nm using a UV–visible spectrophotometer, and the phenolic content of the extract was estimated using a calibration curve ($y = 0.0054x + 0.3845$), with GA standard. The findings were reported as milligrams of gallic acid equivalents (GAE) per gram of extract. This process was carried out three times at every concentration.

2.6 | Determination of Total Flavonoid Content

The flavonoid content was quantified using the aluminum chloride modified method as described in Seifu et al. [23]. In 1 mL of the extract sample in a test tube, 4 mL of distilled water

and 0.3 mL of 5% NaNO₂ were mixed. After 5-min incubation, 0.3 mL of 10% AlCl₃ was added. One minute later, 2 mL of 1 M NaOH was incorporated, and the final volume was adjusted to 10 mL using distilled water, followed by 15-min ambient retention. Quercetin was used as a standard. The extract was evaluated at 1000 µg/mL concentration. Absorbance of the sample was measured at 510 nm and compared against the quercetin standard. The total flavonoid content was expressed as milligrams of quercetin equivalent (mg QE/g) per gram of extract. This process was carried out three times at every concentration.

2.7 | Total Tannin Content Assay

To 0.1 mL of the extract samples in test tubes, 7.5 mL of distilled water, 0.5 mL of FCR, and 1 mL of 35% Na₂CO₃ were introduced. After adjusting the solution volume to 10 mL with distilled water, each mixture was vortexed for 15 s, and for 30 min at ambient conditions. GA was used as a standard. The extract was evaluated at 1000 µg/mL concentration. The absorbance of mixtures was measured at 725 nm and compared with standard GA [24]. This process was carried out three times at every concentration.

2.8 | Total Antioxidant Capacity (TAC)

In separate test tubes, 0.5 mL of sample solution at different concentrations and 5 mL of phosphomolybdate reagent were mixed thoroughly. Following incubation for 90 min at 95°C, the mixtures were allowed to cool to room temperature and the absorbance was recorded at 695 nm. We used AA as a standard. Standard ascorbic acid equivalent (AAE) was used to estimate the antioxidant capacity [25, 26]. All the test samples (extracts & standard) were evaluated at six different concentrations, which are 200, 100, 50, 25, 12.5, and 6.25 µg/mL. This process was carried out three times at every concentration.

2.9 | Animals

For this study, Swiss-albino mice weighing about 25–40 g were maintained at a temperature of 24°C ± 1°C, a relative humidity of 55%–65%, and a 12-h light–dark cycle at the physiology laboratory at R. P. Shaha University, Narayanganj-1400, Bangladesh, to ensure standard conditions. The animals were supplied with mouse diet manufactured by ICDDR,B and water. The animals were acclimated to experimental conditions for 1 week, and the experimental protocol was approved by the Animal Ethics Committee of the R. P. Shaha University (Reference number: RPSU/EC/Phr/2025/02).

2.10 | Oral Glucose Tolerance Test (OGTT)

2.10.1 | Experiment Design

Twenty male mice were randomly distributed into four groups ($n = 5$ per group) labeled Group I, Group II, Group III, and Group IV, with each group having five mice. Group I was administered 2% aqueous Tween 80 (10 mL/kg weight), designated as the control group. Group II was administered glibenclamide at 5 mg/kg dose and designated as the standard group. Groups III and IV received the nHE of the leaves of *H. undatus* at dosages of 250 mg/kg and 500 mg/kg body weight, designated as Test I and Test II groups, respectively. Before treatment, each mouse was

weighed, and the doses of the test samples and standard were adjusted correspondingly.

2.10.2 | Procedure

After 12-h fasting, the sample and drug were given to mice. After 1 h, all mice were given a glucose load (2 g/kg), and blood glucose levels were measured at 0, 30, 60, 90, and 120 min after glucose loading using a glucose meter [27].

2.11 | Antidiarrheal Test

2.11.1 | Experiment Design

Twenty male mice were randomly categorized into four groups ($n = 5$ per group) labeled Group I, Group II, Group III, and Group IV with each group including five mice. These groups had specific treatments: control, standard, and two doses of the nHE (250 mg/kg, and 500 mg/kg), and the doses of all test materials were adjusted based on the weight of individual mouse.

2.11.2 | Procedure

The mice were administered the samples one hour before the oral delivery of castor oil, which was given at a dose of 0.5 mL per mouse for 30 g body weight (16.7 mL/kg). Each individual mouse from each group was housed in a separate cage coated with blotting paper on the floor to assess the occurrence of diarrhea hourly for four hours following the oral delivery of castor oil. The quantity of feces or any fluid substance that stained the blotting paper was recorded hourly during a 4-h period for each mouse. The latent period for each mouse was recorded. At the start of each hour, new blotting sheets were substituted for the old ones [28, 29].

2.11.3 | Gas Chromatography–Mass Spectrometry (GC–MS)

The phytochemical analysis of the nHE of *H. undatus* was performed by using a GC–MS (Agilent 19091S-433UI:2489267H, USA). Splitless injection of 1 µL of extract was made into the injection port of the gas chromatography instrument maintaining the input temperature at 250°C; the oven temperature was first set to 60°C for 0 min, thereafter increasing to 240°C at a rate of 5°C per minute over a duration of 4 min. The total duration was 40 min. Carrier gas helium was set at 1.0 mL/min steady flow together with the temperature of the interface transfer line at 280°C. Mass spectrometry detection was configured in scan mode. The temperatures of the quadrupole analyzer and ion source were 230°C and 150°C, respectively. Ions were generated using electron ionization (EI) at 70 eV. The scanning duration and mass range were 1 s and 50–550 m/z, respectively [30].

2.11.4 | Protein and Ligand Preparation

For this study, the 3D crystal structure of α -amylase enzyme (PDB ID: 3DHP) [31] and the selected ligands (SDF format) were retrieved from the RCSB Protein Data Bank (<https://www.rcsb.org>) and the PubChem database, respectively. These ligand structures were optimized through energy minimization and protonation using Avogadro software, followed by conversion to PDB format for docking studies [32]. Using BIOVIA Discovery Studio Visualizer, α -amylase structure was refined by removing

unnecessary chains, heteroatoms, and water molecules. The cleaned protein was then transformed to PDBQT format using Open Babel software.

2.11.5 | Molecular Docking

AutoDock Vina from PyRx was employed to conduct all docking calculations on the active sites of the α -amylase enzyme, using the identified molecules from *H. undatus* [33]. A grid box was selected to cover the maximum surface area of the prepared protein. The grid center points were set to $X=8.5075$, $Y=59.7696$, and $Z=18.8518$, and the dimensions (Å) were $X=73.7025$, $Y=88.0321$, $Z=58.6921$. The magnitudes of the grid box were strategically defined to enclose the active site of the protein, specifically targeting its substrate-binding domain. Visualization was performed using BIOVIA Discovery Studio [32].

2.11.6 | Pharmacokinetic Properties Analysis

Swiss ADME and pkCSM-pharmacokinetics were the online tools used to predict the molecular properties and pharmacokinetics (ADME) of the molecules identified from *H. undatus* [34]. During the generation phase, the Simplified Molecular Input Line Entry System (SMILES) was utilized for the selected molecules [35].

2.11.7 | Toxicological Properties

In addition to ADME analysis, it is crucial to measure the probable toxicity of chemicals on human organs or cells. The toxicity levels of the chosen compounds were estimated using the ProTox-III online server [36].

2.11.8 | Statistical Analysis

All experimental data were analyzed using GraphPad Prism (Version 8.0). Results are reported as mean $\pm$ SEM. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison posttest was used to evaluate the significance between the test samples. A p value less than 0.05 was considered statistically significant.

3 | Result

3.1 | Phytochemical Screening

Initial phytochemical screening of nHE ascertained the presence or absence of carbohydrates, alkaloids, tannins, flavonoids, saponins, gums, steroids, glycosides, xanthoproteins, phenolic compounds, and acidic substances, as shown qualitatively in Table 1.

3.2 | Determination of Total Phenolic and Total Flavonoid Content

To measure total phenolic and total flavonoid content of nHE, standard calibration curves of GA and quercetin were utilized, respectively, and the results were expressed as milligram equivalent per gram of dried sample for both gallic acid (GAE) and quercetin (QE). Based on the calibration equations for total phenolics ($y = 0.0054x + 0.3846$, $R^2 = 0.9994$) and flavonoids ($y = 0.0004x - 0.0192$, $R^2 = 0.9801$), the phenolic content of nHE

was found to be 29.73 ± 0.33 mg GAE/g, while the flavonoid content was 69.3 ± 6.61 mg QE/g. These values suggest that the extract has antioxidant potential.

3.3 | Total Tannin Content Assay

The quantity of tannin in nHE of *H. undatus* leaves was measured using standard GA as milligrams of GAE per gram of dried sample. Based on the calibration equations ($y = 0.0013x + 0.1621$, $R^2 = 0.9908$), the total tannin content of nHE was found to be 14.35 ± 0.221 mg GAE/g.

3.4 | TAC

Phosphomolybdate-based TAC of *H. undatus* was measured using AA standard. From the calibration curve ($y = 0.001x - 0.0065$, $R^2 = 0.9954$), the antioxidant capacity of the extract was 115.58 ± 1.43 mg AAE/g, indicating substantial antioxidant potential and stated as milligrams of AAE per gram of dried sample.

3.5 | OGTT

In 30 min of oral ingestion of glucose, the blood glucose levels were abruptly changed to higher levels among the diabetic mice than the normal control group, as shown in Figure 1. After 30 min, blood glucose levels gradually fallen in the nHE 250 mg/kg and 500 mg/kg groups compared to the diabetic control group. Extract at 500 mg/kg during test produced a marked decrease in blood glucose levels by 14.6% at 30 min, 26.4% at 60 min, 40.9% at 90 min, and 49.4% at 120 min, compared to the corresponding initial level.

3.6 | Antidiarrheal Test

In the mouse model, the nHE of *H. undatus* significantly extended the commencement of diarrhea at two doses, and the highest dose had a superior effect compared to the negative control. Similarly, compared to the control group, the extract at doses of 250 mg/kg, and 500 mg/kg produced a significant decrease in total fecal yield relative to the control group. The results indicated that the percentage inhibition of diarrhea was 14.58% and 39.58% at 250 mg/kg and 500 mg/kg doses, respectively, while standard loperamide (3 mg/kg) showed 58.33% inhibition (Figures 2(a) and 2(b)).

3.7 | GC-MS Analysis

The GC-MS investigation offered a comprehensive overview of the chemical constituents existing in the nHE extract. The chromatographic spectra, depicted in Figure 3, display well-defined peaks that represent the individual compounds detected. E,E,Z-1,3,12-Nonadecatriene-5,14-diol (RT 18.705, % of area 11.91) was most abundant, followed by Lup-20(29)-en-3-one (RT 39.342, 11.53), 9-octadecynoic acid, methyl ester (RT 18.599, 11.31), methyl 14-methyl-eicosanoate (RT 15.883, 7.98), β -sitosterol acetate (RT 34.051, 4.07), γ -sitosterol (RT 37.98, 3.91), and 9-octadecenamide, (Z)-(RT 23.052, 3.1). GC-MS analysis revealed the presence of several major classes of phytochemicals, predominantly fatty alcohols, triterpenoids, fatty acid methyl esters, sterols/phytosterols, and fatty amides. Among the identified compounds, E,E,Z-1,3,12-nonadecatriene-5,14-diol

TABLE 1 | Qualitative tests of nHE of *H. undatus*.

Phytoconstituents	Test used	Observation	Result
Carbohydrate	Molisch's test	Bluish-violet ring	++
Alkaloid	Dragendorff's test	Orange-brown or red precipitate	+
Tannins	Ferric chloride test	Brownish-green color	+
Flavonoids	Shinoda test	Pink color	+
Saponin	Foam test	Foam form	-
Gums	Molisch's test	Red violet ring	-
Steroids	Liebermann–Burchard's test	Greenish color	++
Glycoside	Keller–Kiliani test	Reddish-brown ring	-
Xanthoproteins	Yellow protein test	Yellow/orange	-
Phenolic compounds	FeCl ₃ solution test	Dark green/bluish-black	++
Acidic compounds	Sodium bicarbonate test	Brisk effervescence (bubbles)	+

Note: (–) = not present; (+) = present; (++) = highly present.

was classified as a fatty alcohol, while Lup-20(29)-en-3-one represented a triterpenoid component. The fatty acid-derived constituents were mainly present as fatty acid methyl esters, including 9-octadecynoic acid, methyl ester, and methyl 14-methyl-eicosanoate. Steroidal constituents were also detected, with β -sitosterol acetate belonging to the broader class of steroids and γ -sitosterol identified as a phytosterol. Additionally, (Z)-9-octadecenamide was identified as a fatty amide. Collectively, fatty acid-derived compounds constituted the major proportion of the extract, followed by sterols/triterpenoids, indicating a lipid-rich phytochemical profile. S1 Table further supplements the GC–MS findings by presenting detailed information for each identified compound, including retention time and peak area. These data provide a thorough characterization of the bioactive constituents detected in the nHE extract.

3.8 | Molecular Docking

Molecular docking was performed employing the crystal structure of α -amylase (PDB ID: 3DHP). The binding energies of all compounds, obtained from the docking analysis, are summarized in Table 2. The noncovalent interactions between 3DHP and the 20 selected compounds, along with the 3DHP–voglibose complex, are also illustrated in Figure 4. The molecular docking analysis revealed that β -amyronone exhibited the highest binding affinity toward the 3DHP enzyme, with a docking score of -9.7 kcal/mol. Campesterol, stigmasterol, and lupeol also showed strong interactions, with docking scores of -9.6 , -9.6 , and -9.4 kcal/mol, respectively. In contrast, the synthetic drug voglibose demonstrated a comparatively lower binding affinity, with a docking score of -6.2 kcal/mol.

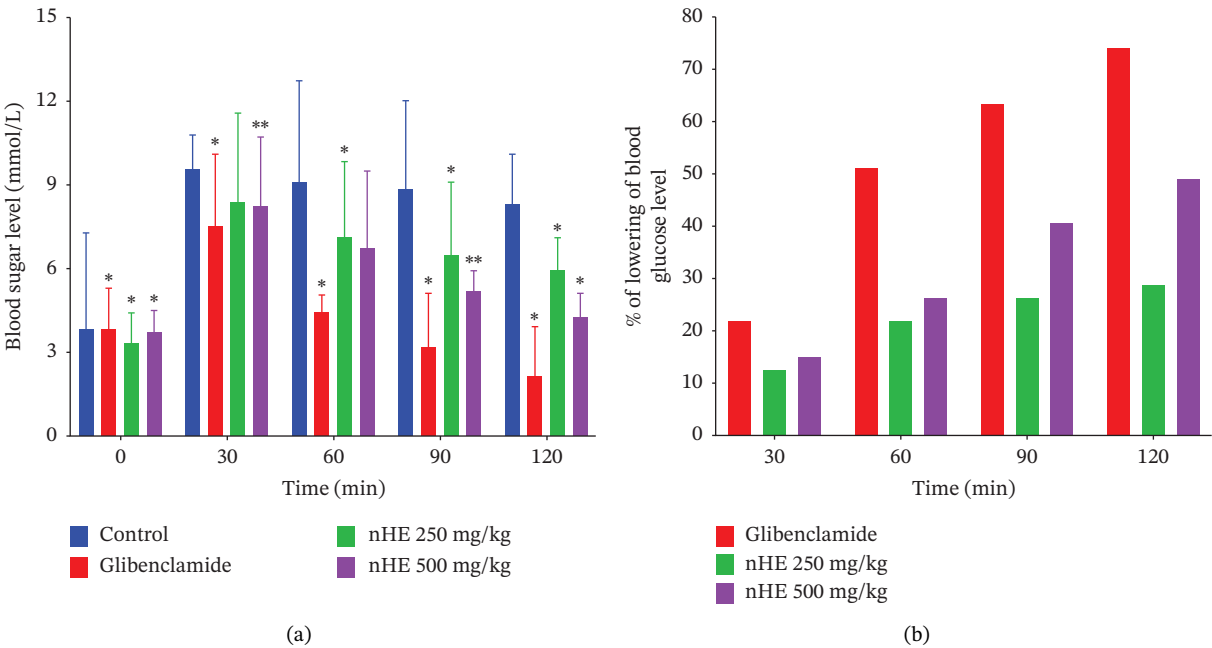


FIGURE 1 | (a) Oral glucose tolerance test (OGTT) and (b) % of reduction of blood glucose level of nHE of *H. undatus*. Data are reported as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ compared to the control group ($n = 5$ animals per group).

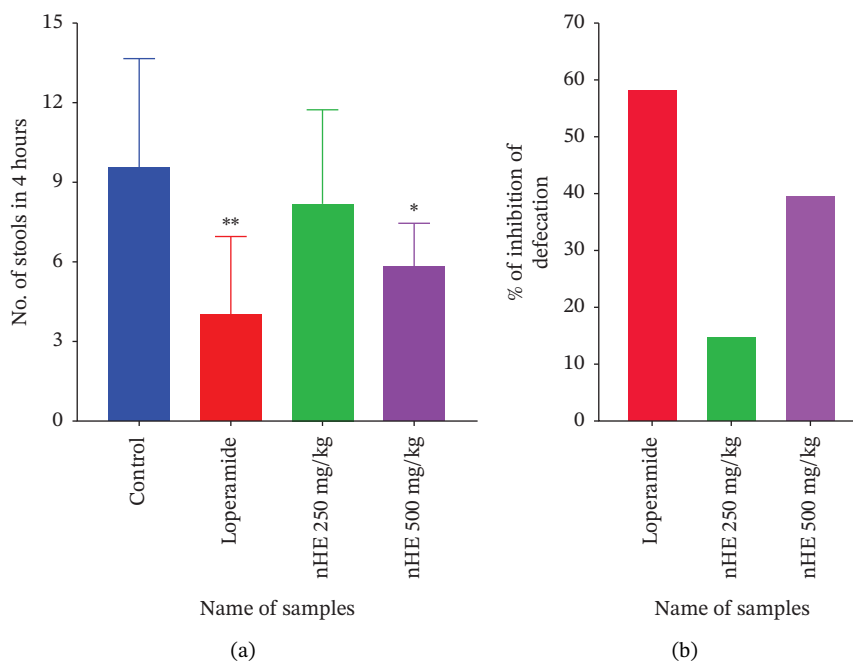


FIGURE 2 | (a) Effect of *H. undatus* extract and standard on stool in castor oil-induced diarrheal episode in mice and (b) effect of *H. undatus* extract on the basis of % inhibition of defecation in castor oil-induced diarrheal episode in mice. Values are mean ± SEM ($n = 5$). * $p < 0.05$, ** $p < 0.01$ compared to the control group ($n = 5$ animals per group).

Overall, β -amyrone, campesterol, stigmasterol, and lupeol emerged as the most promising natural candidates for inhibiting the α -amylase enzyme.

SwissADME generates a drug-likeness radar chart (S1 Figure), where each axis represents a key property, and the pink area marks the optimal range. The physicochemical and ADMET characteristics of the compounds are concise in Tables 3 and 4, supporting their suitability for drug development. Additionally, the pKCSM server was employed to perform a complementary ADMET analysis.

3.9 | Toxicity Predictions

In this study, none of the 20 selected compounds exhibited hepatotoxicity, cytotoxicity, or mutagenicity, as shown in Table 5. These findings suggest that 20 compounds possess favorable safety profiles and oral bioavailability, highlighting their potential as hopeful drug entities for further development prospects.

4 | Discussion

The current research comprehensively explored the pharmacological and phytochemical properties of *H. undatus* leaves nHE,

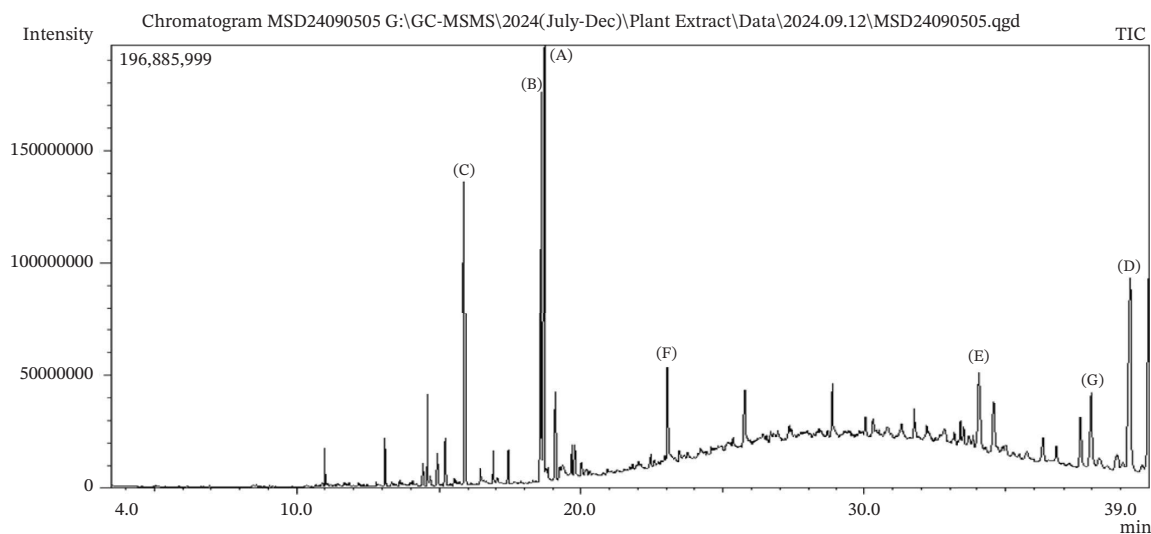


FIGURE 3 | GC-MS chromatogram of *n*-hexane extract of the leaf of *H. undatus*. The major peaks were annotated with the identified dominant compounds: (A) E,E,Z-1,3,12-Nonadecatriene-5,14-diol, (B) Lup-20(29)-en-3-one, (C) 9-octadecynoic acid, methyl ester, (D) methyl 14-methyl-eicosanoate, (E) β -sitosterol acetate, (F), γ -sitosterol, and (G) 9-octadecenamide, (Z)-.

TABLE 2 | Binding affinity scores of identified molecules docked into α -amylase enzyme.

S. N.	Compound name	PubChem CID	Binding affinity (kcal/mol)
1	Voglibose	444,020	−6.2
2	β -Amyrone	12,306,160	−9.7
3	Campesterol	173,183	−9.6
4	Stigmasterol	5,280,794	−9.6
5	Lupeol	259,846	−9.4
6	9,19-Cyclolanost-25-ene-3,24-diol	11,419,367	−9.2
7	Ergost-5-en-3-ol, acetate, (3 β .,24R)	13,019,955	−9.1
8	Lupeol, trifluoroacetate	91,704,083	−9.1
9	Cholesta-4,6-dien-3-ol, (3 β .)	14,795,191	−8.8
10	Lup-20 (29)-en-3-ol, acetate, (3 β .)	92,157	−8.8
11	β -Sitosterol acetate	5,354,503	−8.6
12	γ -Sitosterol	457,801	−8.6
13	Cholest-7-en-3-ol, 14-methyl-, (3 β .)	21,125,985	−8.4
14	Vitamin E	14,985	−8.4
15	γ -Tocopherol	92,729	−8.1
16	1,1,6-Trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane	91,700,552	−7.7
17	Squalene	638,072	−7.5
18	Lup-20 (29)-en-3-one (2)	323,075	−7.4
19	α -Tocopherolquinone	24,205	−7.3
20	4,8,12,16-Tetramethylheptadecan-4-olide	567,149	−6.5
21	2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane	91,694,785	−6.5

proving its various therapeutic potential by an integrated in vitro, in vivo, and in silico method. Qualitative phytochemical screening established the presence of a diverse array of bioactive components like flavonoids, alkaloids, tannins, steroids, glycosides, saponins, phenolics, and carbohydrates—the majority of which have a wide range of beneficial effects including antioxidant, antidiabetic, and gastroprotective activities [37]. Flavonoids and phenolic compounds are well known for their free radical scavenging and metal chelating properties, which can mitigate oxidative stress, a key pathological factor in the progression of diabetes, and gastrointestinal disorders. These compounds may also enhance endogenous antioxidant defenses and modulate carbohydrate metabolism by inhibiting digestive enzymes such as α -amylase [38, 39]. Alkaloids and saponins have been reported to exert antidiabetic effects via improvement of insulin sensitivity, modulation of glucose uptake, and suppression of intestinal glucose absorption, while tannins may reduce postprandial hyperglycemia by forming complexes with digestive enzymes and dietary carbohydrates [40, 41].

Additionally, steroids and glycosides have been associated with anti-inflammatory and cytoprotective effects, which may contribute to gastrointestinal mucosal protection by stabilizing cell membranes and reducing oxidative and inflammatory damage [42, 43]. The results were further substantiated by quantitative assays [44]. The observed antioxidant potential of the extract corroborates earlier findings that plant-derived flavonoids and phenolic compounds play a central role in mitigating oxidative stress through effective scavenging of ROS. Previous studies have

consistently demonstrated that polyphenol-rich extracts exhibit strong antioxidant properties, which are crucial in preventing oxidative damage to biomolecules such as DNA, proteins, and lipids. Given that oxidative stress is a key contributor to the development and progression of diabetes mellitus, the antioxidant profile of the extract supports its therapeutic relevance in metabolic disorders [45]. Similar antioxidant trends have been reported for related medicinal plants, reinforcing the role of flavonoids and phenolic acids in redox regulation.

The hypoglycemic activity observed in vivo is in agreement with prior experimental studies reporting significant glucose-lowering effects of polyphenol-rich plant extracts in diabetic animal models. Earlier investigations have shown that flavonoids and phenolic acids can modulate glucose homeostasis by improving insulin sensitivity, enhancing peripheral glucose uptake, and inhibiting carbohydrate-digesting enzymes such as α -amylase and α -glucosidase. Additionally, certain phenolic constituents are known to protect pancreatic β -cells and promote insulin secretion. The present findings are therefore consistent with the existing literature, suggesting that the antihyperglycemic effect of the extract is likely mediated through a synergistic interaction of its bioactive polyphenolic constituents rather than a single dominant compound [46, 47]. To rationalize this effect, in silico molecular docking studies were employed. The in silico molecular docking studies proved that certain of the nHE phytoconstituents possess decent binding capacities to the α -amylase enzyme (PDB ID: 3DHP), a crucial enzyme used in the splitting of starch within the gastrointestinal tract. The reported binding

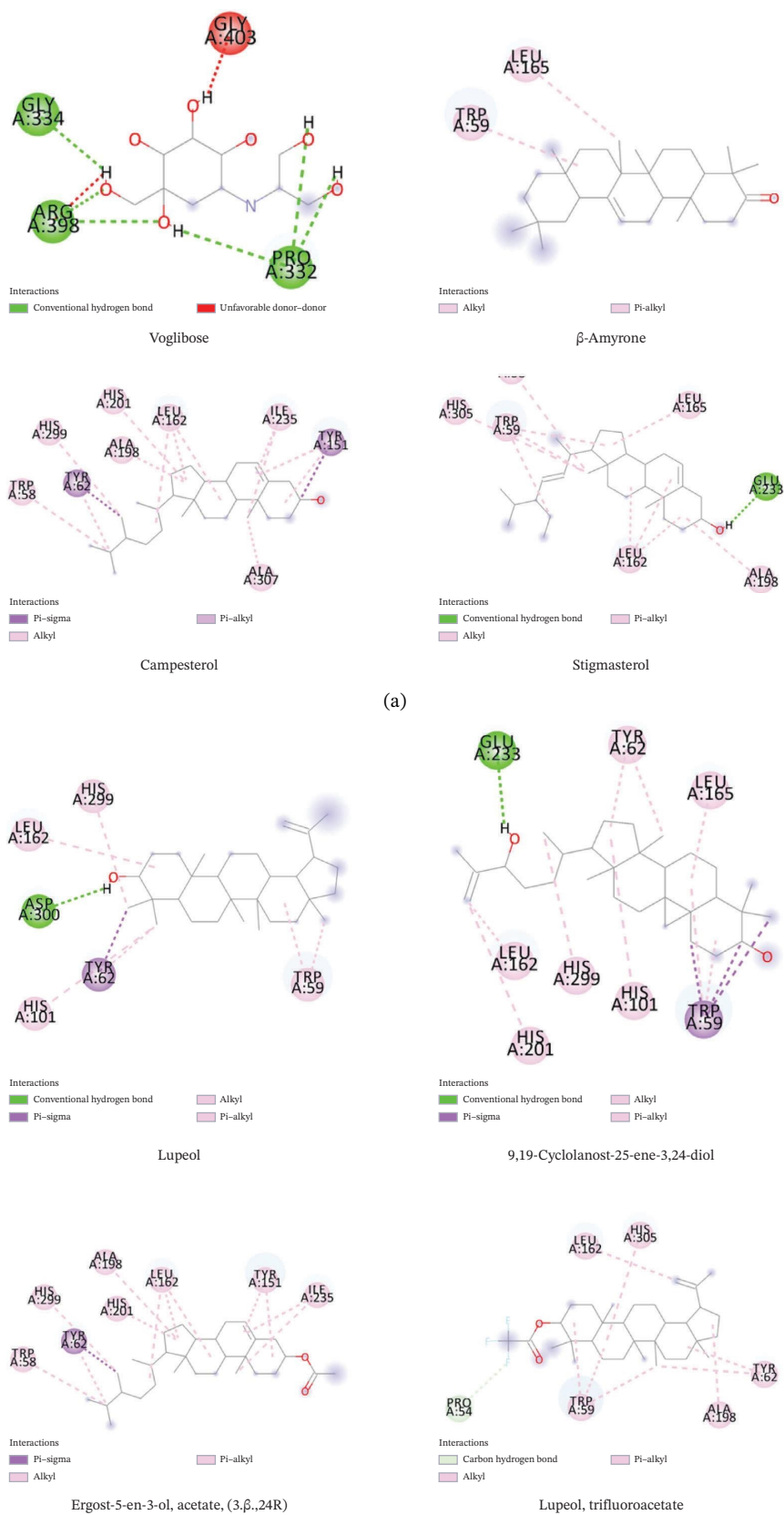


FIGURE 4 | (Continued)

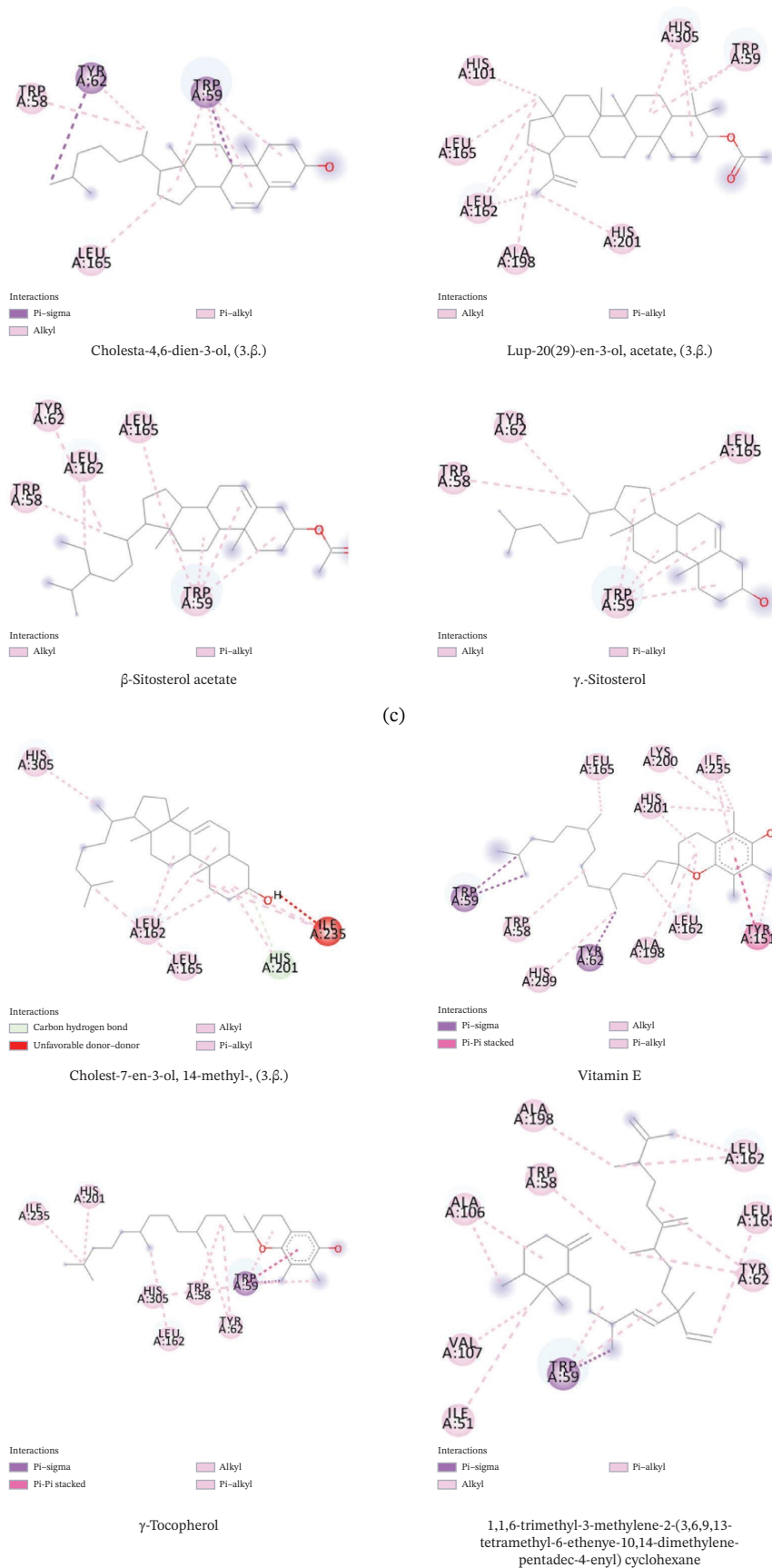


FIGURE 4 | (Continued)

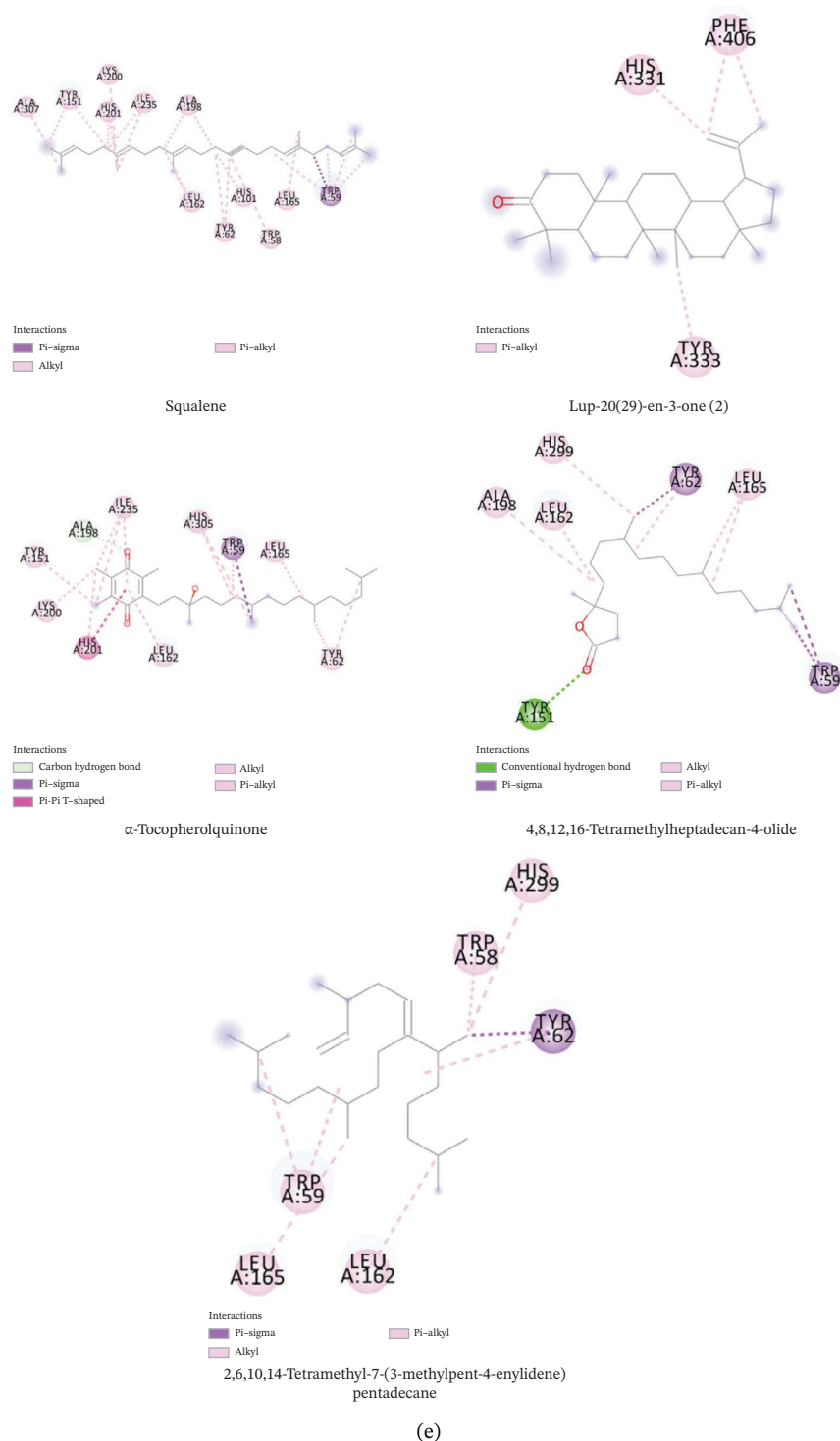


FIGURE 4 | 2D interaction of reference drug and selected 20 molecules.

energies reveal the potential of such compounds to suppress α -amylase activity, thereby slowing carbohydrate digestion and glucose absorption, an accepted therapeutic regimen for controlling postprandial hyperglycemia in Type 2 diabetes [48].

However, it is important to emphasize that molecular docking provides only a theoretical estimation of ligand enzyme interactions under static conditions. Docking scores reflect predicted binding energies and do not account for protein flexibility,

solvent effects, metabolic transformations, or in vivo pharmacokinetic behavior, which may significantly influence biological activity. Therefore, the docking results should be interpreted as supportive rather than confirmatory evidence of α -amylase inhibition.

Importantly, a structure–activity relationship (SAR) trend was observed among the docked compounds. Molecules containing pentacyclic triterpenoid skeletons (such as β -amyrone and

TABLE 3 | Molecular properties of molecules and reference drug.

Ligand name	Molecular weight g/mol	Num. of H-bond acceptors	Num. of H-bond donors	Fraction Csp3	TPSA (Å ²)	Log Po/w	Log S (ESOL)	Lipinski rule	Bioavailability score
Voglibose	267.28	8	8	1.00	153.64	0.88	1.41	Yes; 1 violation	0.55
β -Amyrone	424.70	1	0	0.90	17.07	4.51	-8.04	Yes; 1 violation	0.55
Campesterol	400.68	1	1	0.93	20.23	4.97	-7.54	Yes; 1 violation	0.55
Stigmasterol	412.69	1	1	0.86	20.23	5.08	-7.46	Yes; 1 violation	0.55
Lupeol	426.72	1	1	0.93	20.23	4.72	-8.64	Yes; 1 violation	0.55
9,19-Cyclolanost-25-ene-3,24-diol	442.72	2	2	0.93	40.46	4.94	-7.67	Yes; 1 violation	0.55
Ergost-5-en-3-ol, acetate, (3, β ,24R)-	442.72	2	0	0.90	26.30	5.54	-8.03	Yes; 1 violation	0.55
Lupeol, trifluoroacetate	522.73	5	0	0.91	26.30	4.95	-10.10	No; 2 violations	0.17
Cholesta-4,6-dien-3-ol, (3, β)-	384.64	1	1	0.85	20.23	4.77	-7.17	Yes; 1 violation	0.55
Lup-20 (29)-en-3-ol, acetate, (3, β)-	468.75	2	0	0.91	26.30	5.17	-9.13	Yes; 1 violation	0.55
β -Sitosterol acetate	456.74	2	0	0.90	26.30	5.30	-8.39	Yes; 1 violation	0.55
γ -Sitosterol	414.71	1	1	0.93	20.23	5.07	-7.90	Yes; 1 violation	0.55
Cholest-7-en-3-ol, 14-methyl-, (3, β)-	400.68	1	1	0.93	20.23	4.97	-7.56	Yes; 1 violation	0.55
Vitamin E	430.71	2	1	0.79	29.46	6.04	-8.60	Yes; 1 violation	0.55
γ -Tocopherol	416.68	2	1	0.79	29.46	5.16	-8.29	Yes; 1 violation	0.55
1,1,6-Trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane	452.80	0	0	0.70	0.00	6.51	-9.75	Yes; 1 violation	0.55
Squalene	410.72	0	0	0.60	0.00				
Lup-20 (29)-en-3-one (2)	424.70	1	0	0.90	17.07	4.54	-8.43	Yes; 1 violation	0.55
α -Tocopherolquinone	446.71	3	1	0.79	54.37	5.83	-7.14	Yes; 1 violation	0.55
4,8,12,16-Tetramethylheptadecan-4-olide	324.54	2	0	0.95	26.30	4.15	-6.01	Yes; 1 violation	0.55
2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane	348.65	0	0	0.84	0.00	5.46	-7.94	Yes; 1 violation	0.55

TABLE 4 | ADMET study of molecules and reference drug.

Ligand no.	Absorption		Distribution		Metabolism		Excretion		Toxicity	
	Water Solubility (log mol/L)	Human intestinal absorption (%)	BBB permeability (log BB)	CNS Permeability (log PS)	CYP3A4 substrate	CYP3A4 inhibitor	Total Clearance (log mL/min/kg)	Renal OCT2 substrate	Human max. Tolerated dose (log mg/kg/day)	Oral rat acute toxicity, LD50 (mol/kg)
Voglibiose	−1.153	23.152	−0.927	−4.673	No	No	0.938	No	0.946	1.023
β -Amyrone	−6.674	97.473	0.693	−1.773	Yes	No	−0.096	No	−0.31	2.261
Campesterol	−6.818	94.757	0.771	−1.805	Yes	No	0.572	No	−0.641	2.28
Stigmasterol	−6.682	94.97	0.771	−1.652	Yes	No	0.618	No	−0.664	2.54
Lupeol	−5.861	95.782	0.726	−1.714	Yes	No	0.153	No	−0.502	2.563
9,19-Cyclolanost-25-ene-3,24-diol	−5.626	93.699	−0.357	−1.681	Yes	No	0.344	No	0.344	3.921
Ergost-5-en-3-ol, acetate, (3, β ,24R)-	−7.02	96.869	0.731	−1.845	Yes	No	0.495	No	−0.524	2.23
Lupeol, trifluoroacetate	−6.053	95.206	0.6	−1.892	Yes	No	−0.202	No	−0.432	2.874
Cholesta-4,6-dien-3-ol, (3, β)-	−6.608	94.871	0.779	−1.76	Yes	No	0.597	No	−0.71	2.282
Lup-20 (29)-en-3-ol, acetate, (3, β)-	−6.006	97.894	0.644	−1.754	Yes	No	0.06	No	−0.41	2.512
β -Sitosterol acetate	−6.936	96.576	0.749	−1.818	Yes	No	0.551	No	−0.476	2.333
γ -Sitosterol	−6.773	94.464	0.781	−1.705	Yes	No	0.628	No	−0.621	2.552
Cholest-7-en-3-ol, 14-methyl-, (3, β)-	−6.758	93.548	0.752	−1.795	Yes	No	0.536	No	−0.73	2.32
Vitamin E	−6.901	89.782	0.876	−1.669	Yes	No	0.794	No	0.775	2.072
γ -Tocopherol	−7.602	90.043	0.739	−1.669	Yes	No	0.821	No	0.781	2.21
1,1,6-Trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane	−8.53	95.364	1.051	−1.407	Yes	No	1.259	No	0.085	1.807
Squalene	−8.401	89.002	0.965	−0.935	Yes	No	1.791	No	−0.533	1.893
Lup-20 (29)-en-3-one (2)	−5.828	98.467	0.751	−1.568	Yes	No	0.102	No	0.008	2.556
α -Tocopherolquinone	−6.704	94.435	−0.365	−2.093	Yes	No	1.146	No	0.131	1.9
4,8,12,16-Tetramethylheptadecan-4-olide	−7.322	93.397	0.747	−1.875	Yes	No	1.155	No	0.244	1.726
2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene)pentadecane	−8.671	93.341	1.038	−0.953	Yes	No	1.604	No	−0.131	1.686

TABLE 5 | Toxicity profiling of seven compounds with reference drug.

Ligand name	Hepatotoxicity	Carcinogenicity	Mutagenicity	Cytotoxicity
Voglibose	Inactive	Inactive	Inactive	Inactive
β -Amyrone	Inactive	Inactive	Inactive	Inactive
Campesterol	Inactive	Inactive	Inactive	Inactive
Stigmasterol	Inactive	Inactive	Inactive	Inactive
Lupeol	Inactive	Inactive	Inactive	Inactive
9,19-Cyclolanost-25-ene-3,24-diol	Inactive	Inactive	Inactive	Inactive
Ergost-5-en-3-ol, acetate, (3 β ,24R)-	Inactive	Active	Inactive	Inactive
Lupeol, trifluoroacetate	Inactive	Inactive	Inactive	Inactive
Cholesta-4,6-dien-3-ol, (3 β)-	Inactive	Inactive	Inactive	Inactive
Lup-20 (29)-en-3-ol, acetate, (3 β)-	Inactive	Inactive	Inactive	Inactive
β -Sitosterol acetate	Inactive	Active	Inactive	Inactive
γ -Sitosterol	Inactive	Inactive	Inactive	Inactive
Cholest-7-en-3-ol, 14-methyl-, (3 β)-	Inactive	Inactive	Inactive	Inactive
Vitamin E	Inactive	Inactive	Inactive	Inactive
γ -Tocopherol	Inactive	Inactive	Inactive	Inactive
1,1,6-Trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenyl-10,14-dimethylene-pentadec-4-enyl)cyclohexane	Inactive	Inactive	Inactive	Inactive
Squalene	Inactive	Inactive	Inactive	Inactive
Lup-20 (29)-en-3-one (2)	Inactive	Inactive	Inactive	Inactive
α -Tocopherolquinone	Inactive	Inactive	Inactive	Inactive
4,8,12,16-Tetramethylheptadecan-4-olide	Inactive	Inactive	Inactive	Inactive
2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene)pentadecane	Inactive	Inactive	Inactive	Inactive

lupeol) showed higher binding affinities, likely due to their hydrophobic rings enabling strong van der Waals interactions within the α -amylase active site. The presence of carbonyl or hydroxyl groups in these compounds appeared to enhance hydrogen bonding, further stabilizing ligand–enzyme complexes. Conversely, smaller aliphatic molecules exhibited lower docking scores, reflecting weaker binding interactions. These SAR findings suggest that triterpenoid-like frameworks with both hydrophobic cores and limited polar functionalities are structurally favorable for α -amylase inhibition [49]. While these SAR observations offer useful structural insights, experimental enzyme inhibition assays and molecular dynamics simulations are required to validate binding stability and biological relevance under physiological conditions.

Furthermore, antidiarrheal activities of nHE were demonstrated in the castor oil-induced diarrhea model in mice. The extract significantly deferred the time to the start of diarrhea and also reduced the frequency and amount of fecal output dose dependently. The highest dose level of 500 mg/kg exhibited the greatest inhibition rate of 39.58% for diarrhea with significant antidiarrheal potency. This effect can be mediated by a number of mechanisms, including suppression of intestinal hypersecretion, prevention of release of prostaglandin, or modulation of gastrointestinal motility [50]. The flavonoids and tannins present in the extract could be behind this action because these flavonoids have been shown to increase colonic reabsorption of water and electrolytes and to exert antisecretory effects [51]. In addition, the

tannins can form a protein tannin complex in gastrointestinal mucosa, which will render it impermeable to chemical irritation and reduce its peristaltic movement [52].

Further chemical analysis of the nHE by GC–MS detected several low-molecular-weight compounds that comprised terpenes, fatty acids, and other aromatic compounds, which were likely to be the source of the observed bioactivities [53]. The GC–MS analysis of *H. undatus* identified 72 prominent compounds. From these, 20 molecules were selected for further evaluation based on their molecular docking scores with the α -amylase protein where β -amyron stood out with a notably strong binding affinity of -9.7 kcal/mol. Pharmacokinetic properties (ADMET) of the identified compounds were evaluated by using SwissADME and pkCSM tools, and this revealed good absorption, distribution, metabolism, excretion, and toxicity profiles [54]. This prediction depicted oral bioavailability, noncarcinogenicity, low hepatotoxicity, and absence of any strong adverse drug-like features. Interestingly, the toxicity prediction by using the ProTox-III platform revealed that the compounds fall within safety ranges, and this further improved their potential for future pharmacological exploration [55].

The outcomes of this study offer compelling evidence that the nHE of *H. undatus* leaf possesses significant antioxidant, antidiabetic, and antidiarrheal activities. The results thereby present a pharmacological rationale for the ethnic use of this species for curing metabolic and gastrointestinal disorders.

5 | Conclusion

The nHE of the leaf of *H. undatus* shows great pharmacological potential, especially in antioxidant, anti-hyperglycemic, and antidiarrheal activities. These activities could be justified through its great phytochemical contents, especially flavonoids and phenolics. The ADMET predictions and in silico docking further support the extract's bioactive potential and safety. These findings together provide a scientific rationale for the isolation of single compounds, mechanism studies, and development of *H. undatus*-based phytopharmaceuticals for the treatment of oxidative stress-mediated disorders like diarrhea and diabetes.

5.1 | Limitations and Future Directions

Despite the promising findings, this study has certain limitations that should be acknowledged. The biological activities were evaluated using crude plant extracts, which may contain multiple constituents acting synergistically or antagonistically; therefore, the specific compounds responsible for the observed effects could not be identified. Additionally, the study was conducted at limited dose levels, and a comprehensive dose–response analysis was not performed, which restricts precise assessment of potency and efficacy. Future investigations should focus on bioactivity-guided fractionation and isolation of active compounds, detailed dose response studies, and mechanistic evaluations to further validate and strengthen the therapeutic potential of the plant. Furthermore, although GC–MS analysis was employed for phytochemical profiling, the chromatographic output quality was limited, as the analysis was performed at an external facility, and the resolution, peak labeling, and extent of available chromatographic parameters could not be optimized or expanded beyond the information provided.

Nomenclature

<i>H. undatus</i>	<i>Hylocereus undatus</i>
ROS	Reactive oxygen species
nHE	<i>n</i> -Hexane extract
TAC	Total antioxidant capacity
OGTT	Oral glucose tolerance test
GC–MS	Gas chromatography–mass spectrometry

Author Contributions

Conceptualization: Md. Tariqul Islam.

Data curation: Sudipta Saha.

Formal analysis: A. B. M. Ashraful.

Investigation: Md. Tariqul Islam and Md. Shafiqul Islam.

Methodology: Md. Tariqul Islam and Sudipta Saha.

Resources: Md. Tariqul Islam and A. B. M. Ashraful.

Software: A. B. M. Ashraful.

Supervision: Md. Tariqul Islam.

Validation: Md. Tariqul Islam.

Visualization: Md. Tariqul Islam and Md. Shafiqul Islam.

Writing–original draft preparation: A. B. M. Ashraful.

Writing–review and editing: Md. Tariqul Islam and Md. Shafiqul Islam.

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Disclosure

After reviewing, all authors gave their approval for publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*)

Figures S1 and Tables S1 relating to this paper are available online.

Table S1: GC–MS analysis of nHE.

Figure S1: Swiss ADME bioavailability radar of reference drug and selected compound, where the pink areas represent each property (lipophilicity, molecular weight, solubility, and flexibility).